Biochimica et Biophysica Acta 1792 (2009) 847–852

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Familial tumoral calcinosis and the role of O-glycosylation in the maintenance of phosphate homeostasis

Ilana Chefetz [a,c], Eli Sprecher [a,b,c,*]

[a] Center for Translational Genetics, Rappaport Institute for Research in the Medical Sciences, Haifa, Israel
[b] Faculty of Medicine, Technion- Israel Institute of Technology, Haifa, Israel
[c] Department of Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

ARTICLE INFO

Article history:
Received 1 September 2008
Received in revised form 12 October 2008
Accepted 14 October 2008
Available online 25 October 2008

Keywords:
Calcinosis
GALNT3
KLOTHO
FGF23

ABSTRACT

Familial tumoral calcinosis refers to a group of disorders inherited in an autosomal recessive fashion. Hyperphosphatemic tumoral calcinosis is characterized by increased re-absorption of phosphate through the renal proximal tubule, resulting in elevated phosphate concentration and deposition of calcified deposits in cutaneous and subcutaneous tissues, as well as, occasionally, in visceral organs. The disease was found to result from mutations in at least 3 genes: *GALNT3*, encoding a glycosyltransferase termed ppGalNacT3, *FGF23* encoding a potent phosphaturic protein, and *KL* encoding Klotho. Recent data showed that ppGalNacT3 mediates O-glycosylation of FGF23, thereby allowing for its secretion and possibly protecting it from proteolysis-mediated inactivation. Klotho was found to serve as a co-receptor for FGF23, thereby integrating the genetic data into a single physiological system. The elucidation of the molecular basis of HFTC shed new light upon the mechanisms regulating phosphate homeostasis, suggesting innovative therapeutic strategies for the management of hyperphosphatemia in common acquired conditions such as chronic renal failure.

1. Introduction

Extraosseous calcification (calcification occurring in non-osseous tissues) is a well-known complication of many common disorders such as chronic renal failure or hyperparathyroidism which has been shown to predict morbidity and mortality in the general population. [1–5]. Thus, much effort has been invested in an attempt to delineate the mechanisms responsible for maintaining normal circulating phosphate levels. The study of rare monogenic disorders can be instrumental in the discovery of novel physiological pathways [6]. As reviewed in the present article, the deciphering of the molecular basis of familial tumoral calcinosis (FTC) illustrates remarkably well this paradigm.

2. Familial tumoral calcinosis: (at least) two distinct metabolic phenotypes

Calcinosis refers to the ectopic (non-osseous) deposition of calcium salts [7]. Cutaneous calcinosis is a relatively common clinical occurrence, reported in diseases as common as chronic renal failure, atherosclerosis, acne, cancer and autoimmune diseases [8]. *Hereditary* idiopathic calcinosis, known as FTC, was first described more than a century ago by Giard [8]. The exact prevalence rate of FTC is unknown; however, the disease has been mainly reported in patients of Middle Eastern or African ancestry [9]. All forms of FTC are transmitted in an autosomal recessive fashion [10]. Two major FTC types have been described: hyperphosphatemic FTC (HFTC; MIM211900), the topic of the present review, which is characterized by marked and persistent hyperphosphatemia leading to the development of large periarticular calcified masses, weighing up to 1 kg [11,12]; and normophosphatemic FTC (NFTC; MIM610455), featuring smaller calcified masses often located at pressure points but not only [10,13,14]. Visceral calcification can accompany HFTC [15] while it has never been reported in NFTC. In both forms of FTC, renal function is normal. However, in HFTC, intestinal and renal tubular phosphate re-absorption is increased in the face of inappropriately normal levels of parathyroid hormone and vitamin D metabolites, reflecting elevated activity of the major inducible Na/Pi co-transporters, NaPiIIa-c [11,12,16–18]. In contrast, the pathogenesis of ectopic calcification in NFTC is not associated with metabolic abnormalities. Here, the disease seems to result from aberrant regulation of inflammation, as attested by the fact that a vasculitis-like rash often precedes tumor formation [10,13].

3. Molecular genetics of FTC

The genetic etiopathogenesis of FTC started to unfold through the study of a large and highly consanguineous kindred of Druze origin affected with HFTC [10]. The Druzes constitute a small ethnic minority of Arab Moslem origin which assembled in Egypt about half a millennium ago and live today in mountainous areas of the Near East [19]. This population is highly inbred due to the fact that marriages

* Corresponding author. Department of Dermatology Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. Tel.: +972 3 6974287.
E-mail address: elisp@tasmc.health.gov.il (E. Sprecher).

0925-4439/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2008.10.008

Table 1
Mutation spectrum in FTC.

Hyperphosphatemic familial tumoral calcinosis			
Gene	Nucleotide change	Predicted consequence	Reference
GALNT3	c.484C>T c.1524 + 5G>A	p.R162X Splicing error	[22]
GALNT3	c.1524 + 1G>A	p.K465_Y508del	[22]
GALNT3	c.484C>T c.516-2A>T	p.R162X Splicing error	[82]
GALNT3	c.815 C>A c.1076 C>A	p.T272K p.T359K	[43]
GALNT3	c.1774 C>T	Q592X	[83]
GALNT3	c.41-58del	Protein truncation	[66]
GALNT3	C1387 A>T	K463X	[87]
GALNT3	966T>G 1441C>T	Y322X Q481X	[88]
GALNT3	c.1102_1103insT	E375X	[84]
GALNT3	1460 G>A	W487X	[84]
GALNT3	C.516-2A >T	Splicing error	[85]
FGF23	c.211A>G	p.S71G	[50]
FGF23	c.211A>G	p.S71G	[51]
FGF23	c. 386C>T	p.S129F	[49]
FGF23	c.287T>C	p.M96T	[15]
FGF23	c.160C>A	p. Q54K	[52]
Klotho	c.578 A>G	p. H193R	[53]
Hyperostosis hyperphosphatemia syndrome			
Gene	Nucleotide change	Predicted amino acid change	Reference
GALNT3	c. 1524 + 1G>A	p.K465_Y508del	[24]
GALNT3	c. 803-804insC c. 1626 + 1G>A	E271X exon 8 splice site	[25]
GALNT3	c. 1584 G>A	p. R438H	[26]
Normophosphatemic familial tumoral calcinosis			
Gene	Nucleotide change	Predicted amino acid change	Reference
SAMD9	c.4483A>G	p.K1495E	[56]
SAMD9	c. 1031C>T	R344X	[57]

outside the community are forbidden by religious laws and conversion is not allowed [20]. Rare recessive diseases affecting inbred populations are often caused by homozygous mutations, a fact that forms the theoretical basis for a powerful approach to gene localization, termed homozygosity mapping [21]. Using this technique, a splice site mutation in a gene called *GALNT3* was found to segregate with the disease in the affected kindred [22]. Since then, 10 distinct mutations have been identified in families of various origins (Table 1). These mutations have been predicted and, in specific cases, shown, to result in loss of protein expression [23].

Of interest, mutations in *GALNT3* were also found to underlie a bone disease, called hyperphosphatemic hyperostosis syndrome (HHS) [24–26]. Like HFTC, HHS is transmitted in an autosomal recessive fashion and is characterized by hyperphosphatemia [27–31]. The disease manifests with paroxysmal episodes of excruciating pain along the long bones, associated with radiographic evidence of hyperostosis. In one single instance, a founder mutation was found to cause HHS in one ethnic subgroup and HFTC in another [24], strongly suggesting the importance of modifier traits in determining the final expression of deleterious mutations in *GALNT3*.

GALNT3 encodes UDP-N-acetyl-α-d-galactosamine:polypeptide n-acetylgalactosaminyl transferase 3 (ppGalNacT3), one out of 24 members of a family of glycosyltransferases responsible for initiating O-glycosylation [32]. Apart from HFTC and HHS, no other human disease has so far been linked to this group of proteins. Soon after the discovery of mutations in *GALNT3* as the proximal cause of HFTC, it appeared that many individuals displaying typical features of HFTC do not carry mutations in this gene. This observation led a number of investigators to assess other candidate genes of potential relevance to the maintenance of normal circulating levels of phosphate. Circulating phosphate levels are determined by a balance between dietary intake, incorporation in bone and other tissues, and excretion through urine and stool [17,18,33–35]. A large number of Na^+-dependent phosphate-co-transporters are involved in phosphate handling. They have been classified into three different families: type I (SLC17), type II (SLC34) and type III (SLC20). The type II transporters (NaPiIIa, NaPiIIb, and NaPiIIc) are subject to tight regulation and are responsible for the majority of Pi re-absorption in polarized cells. NapiIIb transporter is expressed in the small intestine, lung, mammary glands, testis, and liver [36] and is involved in transcellular transport of phosphate in the small intestine [37]. The NaPiIIa and NaPiIIc transporters are expressed almost exclusively in kidney and control renal phosphate re-absorption [38]. Genetic defects have described for each of these transporters: mutations in *SLC34A1*, coding for NaPiIIa, were reported in two patients with nephrolithiasis or osteoporosis accompanied by hypophosphatemia [39] although these observations are still of a controversial nature [40]; mutations in *SLC34A2* encoding NaPiIIb are associated with pulmonary alveolar microlithiasis (MIM265100); and mutations affecting NaPiIIc function cause hereditary hypophosphatemic rickets with hypercalciuria (HHRH; MIM241530) [41–44]. Although parathyroid hormone and vitamin D3 are potent regulators of phosphate blood levels [33], the fact that they primarily affect calcium homeostasis had suggested the existence of other circulating factors specifically involved in the regulation of phosphate transport. These proteins, known as phosphatonins [35], were discovered through the study of a rare paraneoplastic condition, termed tumor-induced osteomalacia [45], where tumor cells secrete factors increasing the renal excretion of phosphate through the kidney. The best studied among these factors is the fibroblast growth factor 23 (FGF23), which functions by decreasing the expression of the renal phosphate transporter as well as decrease the 1alpha-hydroxylation and increase the (inactivating) 24-hydroxylation of vitamin D [16,46] (Table 2). Gain-of-function mutations in the *FGF23* gene were found to cause dominant hypophosphatemic rickets (MIM193100), a disorder characterized by increased renal tubular excretion of phosphate and bone mass loss [18,47]. Since this disease represents in many aspects the metabolic mirror image of HFTC, some authors [35,48] raised the possibility that HFTC may also be related to defective FGF23 function. And indeed, a year after the identification of mutations in *GALNT3*, a number of laboratories reported loss-of-function in *FGF23* in patients displaying typical HFTC features [15,49–52]. These mutations were found to result in decreased FGF23 stability and/or decreased secretion, as well as reduced (but not absent) activity of FGF23. Finally, a case of atypical HFTC, characterized

Table 2
Phosphate transporters and their diseases.

Protein names	Gene	Expression site	Disease (OMIM)	References
NaPi-I, NPT1	*SLC17A1*	Kidney, liver		
NaPi-IIa	*SLC34A1*	Kidney (proximal tubules; apical) osteoclasts, neurons	Nephrolithiasis or osteoporosis (MIM612286)	[39]
NaPi-IIb	*SLC34A2*	Small intestine, lung, testis, liver, secreting mammary gland	Pulmonary alveolar microlithiasis (MIM265100)	[86]
NaPi-IIc	*SLC34A3*	Kidney (proximal tubules, apical)	Hereditary hypophosphatemic rickets with hypercalciuria HHRH; (MIM241530)	[41–43]
Pit-1 Glvr-1	*SLC20A1*	Ubiquitous		
Pit-2 Glvr-2	*SLC20A2*	Ubiquitous		

by tumoral calcinosis, diffuse osteopenia; sclerosis in the hands, feet, long bones and skull, intracranial calcifications associated with hyperphosphatemia, hypercalcemia and high PTH levels, was found to result from a deleterious mutation in *KL* [53], encoding Klotho, a molecule previously related in mice to senescence [54]. Interestingly, a gain-of-function translocation involving the same gene was shown to cause a reverse phenotype consisting of hypophosphatemic rickets and hyperparathyroidism [55].

As mentioned above, distinctive features seem to demarcate clinically NFTC and HFTC [56]. Accordingly NFTC was found to map to a different locus than HFTC [22] and to result from mutations in a gene termed *SAMD9*, which encodes a 1589 amino acid-long protein of unknown function [56,57]. Given the rarity of NFTC and the fact that the two mutations reported so far in *SAMD9* have been exclusively found in a very small ethnic subgroup, the Jewish Yemenite population, it is very likely that the mutations arouse in this population as a consequence of a selective effect of unknown nature [57]. The two mutations reported so far in *SAMD9* result in loss of expression of this protein. Recent data suggest a role for SAMD9 in the regulation of inflammation, apoptosis and cell proliferation [57,58]. In agreement with these data, TNF-alpha induces SAMD9 expression in a p38 and NFkB-dependant fashion [57].

4. Delineating a novel regulatory pathway through the deciphering of the molecular basis of HFTC

The discovery of at least three genes carrying mutations causing HFTC clearly suggests that the proteins encoded by these genes may function along the same physiological pathway. As mentioned above, ppGalNacT3, a galactosyltransferase encoded by *GALNT3*, is responsible for initiating O-glycosylation usually on serine or threonine residues. More than 20 different ppGalNacTs catalyze the initial enzymatic step of mucin-type O-glycosylation [32]. In this reaction, the monosaccharide *N*-acetylgalactosamine (GalNAc) is transferred to the hydroxyl groups of serine and threonine residues [59]. Galactosyltransferases contain a short cytoplasmic tail, a transmembrane domain, a stem region, a catalytic domain, and a C-terminal lectin domain, unique to this family [60]. The expression of galactosyltransferases, including ppGalNacT3, has been shown to correlate with prognosis in human cancers [61].

Despite the importance of mucin-type-O-glycosylation, as attested by the high degree of conservation of galactosyltransferases throughout evolution [62], HFTC is the only disease in humans known to be caused by mutations in a glycosyltransferase gene (although defective galactosyltransferase activity due to impaired function of a specific chaperone has been shown to cause a rare autoimmune disease called Tn syndrome, which does not manifest clinically, but results in abnormal laboratory findings including thrombocyto- and leukopenia and some signs of hemolytic anemia not warranting any treatment [63]). The very restricted phenotype associated with defective ppGalNacT3 function in humans is suggestive of functional redundancy in tissues unaffected in HFTC. Similarly, mice deficient in ppGalNacT1 only demonstrate altered immune cell trafficking, and moderate or severe bleeding due to the fact that ppGalNAcT1 supports O-glycoprotein expression among a subset of blood coagulation factors [24].

O-glycosylation of FGF23 was shown to be required for its proper secretion in the extracellular milieu [64]. This, of course, immediately raised the possibility that ppGalNacT3 could mediate FGF23 O-glycosylation, a hypothesis that is supported by recent *in vitro* data [65]. ppGalNacT3 was found to selectively direct O-glycosylation of a subtilisin-like proprotein convertase recognition sequence motif; O-glycosylation of this motif results in inhibition of FGF23 proteolytic degradation, pointing to a prime role for ppGalNacT3 in the regulation of FGF23 activity [64]. Accordingly, *FGF23* or *GALNT3* mutations were found to be associated with increased FGF23 proteolysis and concomitantly decreased FGF23 activity [25,50,65,66]. Taken together, these observations suggest that at least two different defects are responsible for the high serum phosphate concentrations typically found in HFTC and HHS: increased proteolysis of FGF23 due to *FGF23* mutations, directly affecting protease recognition site(s), or decreased ppGalNacT3-mediated O-glycosylation of these sites, with consequently enhanced FGF23 susceptibility to proteolytic degradation. Both processes result in decreased phosphaturia as a consequence of decreased levels of active FGF23 activity. Decreased FGF23 activity probably also underlies the pathogenesis of HFTC caused by mutations in *KL*. Indeed, *KL* was found to encode Klotho. Klotho, previously shown in mice to counteract aging in various systems [54], is a multifunctional protein which has been shown to play a major role in the maintenance of calcium homeostasis by influencing the activity of the Na^+, K^+, ATPase pump, driving transepithelial calcium transport, by affecting TRPV5 activity, but also by serving as an essential element of FGF23-mediated signaling [67]. In man, the protein was found to function as a co-receptor for FGF23, converting FGF receptor 1 (IIIc) into a FGF23 receptor [68]. Thus decreased activity of Klotho was also found to result in decreased phosphaturia associated in this case with (possibly compensatory) elevated levels

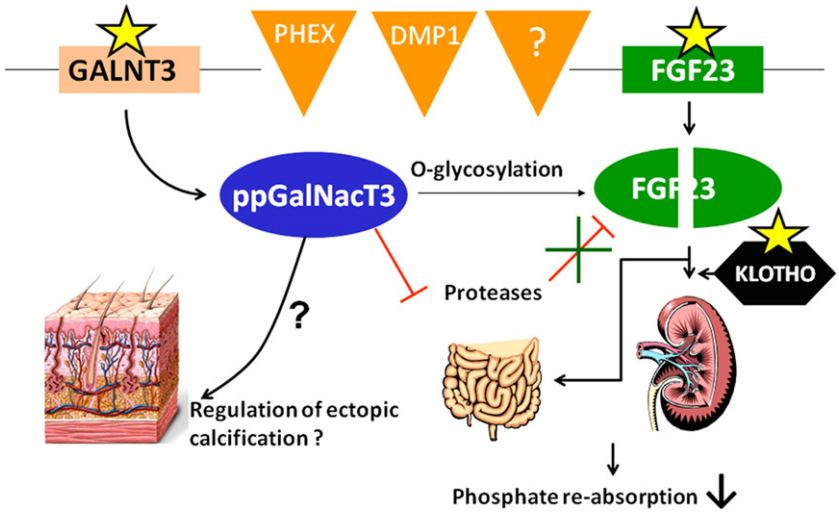

Fig. 1. Role of ppGalNacT3, FGF23 and Klotho in the maintenance of phosphate homeostasis. ppGalNacT3 mediates O-glycosylation of FGF23, thereby protecting it from degradation through proteolysis. Klotho serves as a co-receptor for FGF23. Accordingly, mutations in anyone of these three genes, result in decreased FGF23 activity, increased phosphate re-absorption and consequently, hyperphosphatemia with tumoral calcinosis.

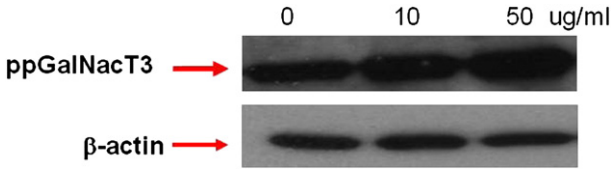

Fig. 2. Doxycycline up-regulates ppGalNacT3 expression in SaOs cells.

of intact FGF23 [53]. Thus, the identification of three genes associated with HFTC reveals the importance of a novel and pivotal regulatory system responsible for maintaining normal circulating concentrations of phosphate (Fig. 1), and therefore possibly involved in many common acquired diseases as already exemplified in a small number of pioneering studies [69,70].

Despite these advances, a number of questions pertaining to the physiological role of ppGalNacT3 remain open. In particular, the fact that calcinosis in HFTC is often confined to cutaneous and subcutaneous tissues suggests that ppGalNacT3 deficiency may have a specific effect on some regional elements in addition to its systemic effect. *GALNT3* is expressed ubiquitously [22], while FGF23 almost exclusively originates from mineralized tissues [71]; these observations are in line with possible additional functions for ppGalNacT3 not related to FGF23. Similarly, the wide range of symptoms manifested by HFTC patients carrying (occasionally identical) loss-of-function mutations in *GALNT3*, point to the existence of additional physiological elements of importance in the regulation of phosphate homeostasis. It is in fact possible that ppGalNacT3 targets other molecules than FGF23, also known to influence phosphate levels such as the various phosphate co-transporters, other phosphatonins or PHEX or DMP1, thought to regulate FGF23 synthesis [38]. Finally, although FGF23 expression has been shown to be regulated by elements of direct relevance to its role in phosphate metabolism [72,73], little is currently known about the elements involved in the physiological control of ppGalNacT3 activity.

5. Treatment of FTC

Given the rarity of the various types of FTC, very few data are currently available regarding the best way to manage these very disabling conditions. What even further adds to the difficulty in interpreting the literature is the fact that many early papers did not made the distinction between NFTC and HFTC. The recent data reviewed above clearly indicate that although phenotypically similar, the two diseases stems from completely different pathophysiological defects. Logically, anti-inflammatory strategies should benefit patients affected with NFTC whereas in HFTC, effective treatment should be designed to target the underlying metabolic defects.

A number of approaches have been tried over the years to treat ectopic calcification, including laser therapy, the use of phosphate-restricted diets, bisphosphonates, calcium channel blockers, corticosteroids, colchicines and chelating agents such as aluminium hydroxide [74–77]. In most cases, results have been disappointing with therapeutic effects being either negligible or short-lasting. A recent report [52] described a good response of a patient with HFTC to a combination of the phosphate binding agent sevelamer with the carbonic anhydrase inhibitor acetazolamide. Of interest, tetracyclines have been shown occasionally to attenuate ectopic calcification [78–80]. A number of hypotheses have been advanced to explain this effect including inhibition of matrix metalloproteinase activity, anti-inflammatory effect, calcium binding or antibacterial action [78]. We were able to show that doxycycline significantly up-regulates ppGalNacT3 expression, suggesting the need to assess the efficacy of tetracyclines in HFTC (Fig. 2). Interestingly, doxycycline was recently shown to exert a beneficial effect (unrelated to its antimicrobial activity) in a mouse model of Marfan syndrome [81], possibly due to its inhibitory effect on MMP activity.

6. Conclusion

The importance of the discovery of the genetic causes of HFTC and NFTC goes much beyond the elucidation of the molecular basis of a fascinating group of genetic diseases. As mentioned in the Introduction, extraosseous calcification has recently attracted much attention because of the recognition of its contribution to morbidity and mortality in the general population. Actually, two forms of ectopic calcification have been recognized in humans: dystrophic calcinosis, where ectopic calcification follows some form of tissue injury (inflammation, cancer, vascular damage etc..) and metastatic calcinosis, which is always associated with an elevated calcium–phosphate product in the circulation (as in renal failure or hyperparathyroidism) [8]. At this regard, NFTC and HFTC nicely recapitulate most features of these two forms of ectopic calcification, and consequently, represent attractive models to design and test innovative therapies of potential relevance to a wide range of common acquired diseases.

Acknowledgements

We are grateful to our patients and their family members for their enthusiastic, and continuous support. We would like also to thank many collaborators for their invaluable contribution to the various studies reviewed above including Orit Topaz, Gabriele Richard, Margarita Indelman, Aryeh Metzker, Ulla Mandel, Mordechai Mizrachi, John G. Cooper, Polina Specktor, Vered Friedman, Gila Maor, Israel Zelikovic, Sharon Raimer, Yoram Altschuler, Mordechai Choder, Dani Bercovich, Jouni Uitto and Reuven Bergman. This work was supported in part by grants provided by the Israel Science Foundation, the Rappaport Institute for Research in the Medical Sciences, Technion, and NIH/NIAMS grant R01 AR052627.

References

[1] N. Boulman, G. Slobodin, M. Rozenbaum, I. Rosner, Calcinosis in rheumatic diseases, Semin. Arthritis Rheum. 6 (2005) 805–812.
[2] T.M. Doherty, L.A. Fitzpatrick, A. Shaheen, T.B. Rajavashisth, R.C. Detrano, Genetic determinants of arterial calcification associated with atherosclerosis, Mayo Clin. Proc. 2 (2004) 197–210.
[3] M.L. Melamed, J.A. Eustace, L. Plantinga, B.G. Jaar, N.E. Fink, J. Coresh, M.J. Klag, N.R. Powe, Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: a longitudinal study, Kidney Int. 2 (2006) 351–357.
[4] M. Tonelli, F. Sacks, M. Pfeffer, Z. Gao, G. Curhan, Relation between serum phosphate level and cardiovascular event rate in people with coronary disease, Circulation 17 (2005) 2627–2633.
[5] R.D. Abbott, H. Ueshima, K.H. Masaki, B.J. Willcox, B.L. Rodriguez, A. Ikeda, K. Yano, L.R. White, J.D. Curb, Coronary artery calcification and total mortality in elderly men, J. Am. Geriatr. Soc. 12 (2007) 1948–1954.
[6] S.E. Antonarakis, J.S. Beckmann, Mendelian disorders deserve more attention, Nat. Rev. Genet. 4 (2006) 277–282.
[7] E. Kostler, H. Porst, U. Wollina, Cutaneous manifestations of metabolic diseases: uncommon presentations, Clin. Dermatol. 5 (2005) 457–464.
[8] D.M. Touart, P. Sau, Cutaneous deposition diseases. Part II, J. Am. Acad. Dermatol. 4 (Pt 1) (1998) 527–544 quiz 545–526.
[9] S. McClatchie, A.D. Bremner, Tumoral calcinosis—an unrecognized disease, Br. Med. J. 5637 (1969) 153–155.
[10] A. Metzker, B. Eisenstein, J. Oren, R. Samuel, Tumoral calcinosis revisited—common and uncommon features. Report of ten cases and review, Eur. J. Pediatr. 2 (1988) 128–132.
[11] R.E. Slavin, J. Wen, D. Kumar, E.B. Evans, Familial tumoral calcinosis. A clinical, histopathologic, and ultrastructural study with an analysis of its calcifying process and pathogenesis, Am. J. Surg. Pathol. 8 (1993) 788–802.
[12] R. Steinherz, R.W. Chesney, B. Eisenstein, A. Metzker, H.F. DeLuca, M. Phelps, Elevated serum calcitriol concentrations do not fall in response to hyperphosphatemia in familial tumoral calcinosis, Am. J. Dis. Child. 8 (1985) 816–819.
[13] G. Gal, A. Metzker, J. Garlick, Y. Gold, S. Calderon, Head and neck manifestations of tumoral calcinosis, Oral Surg. Oral Med. Oral Pathol. 2 (1994) 158–166.
[14] J. Katz, A. Ben-Yehuda, E.E. Machtei, Y.L. Danon, A. Metzker, Tumoral calcinosis associated with early onset periodontitis, J. Clin. Periodontol. 10 (1989) 643–646.
[15] I. Chefetz, R. Heller, A. Galli-Tsinopoulou, G. Richard, B. Wollnik, M. Indelman, F. Koerber, O. Topaz, R. Bergman, E. Sprecher, E. Schoenau, A novel homozygous missense mutation in *FGF23* causes familial tumoral calcinosis associated with disseminated visceral calcification, Hum. Genet. 2 (2005) 261–266.
[16] J. Stubbs, S. Liu, L.D. Quarles, Role of fibroblast growth factor 23 in phosphate homeostasis and pathogenesis of disordered mineral metabolism in chronic kidney disease, Semin. Dial. 4 (2007) 302–308.

[17] X. Yu, K.E. White, FGF23 and disorders of phosphate homeostasis, Cytokine Growth Factor Rev. 2 (2005) 221–232.
[18] S. Fukumoto, Physiological regulation and disorders of phosphate metabolism—pivotal role of fibroblast growth factor 23, Intern. Med. 5 (2008) 337–343.
[19] L.I. Shlush, D.M. Behar, G. Yudkovsky, A. Templeton, Y. Hadid, F. Basis, M. Hammer, S. Itzkovitz, K. Skorecki, The Druze: a population genetic refugium of the Near East, PLoS ONE 5 (2008) e2105.
[20] R. Vardi-Saliternik, Y. Friedlander, T. Cohen, Consanguinity in a population sample of Israeli Muslim Arabs, Christian Arabs and Druze, Ann. Hum. Biol. 4 (2002) 422–431.
[21] V.C. Sheffield, D.Y. Nishimura, E.M. Stone, Novel approaches to linkage mapping, Curr. Opin. Genet. Dev. 3 (1995) 335–341.
[22] O. Topaz, D.L. Shurman, R. Bergman, M. Indelman, P. Ratajczak, M. Mizrachi, Z. Khamaysi, D. Behar, D. Petronius, V. Friedman, I. Zelikovic, S. Raimer, A. Metzker, G. Richard, E. Sprecher, Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis, Nat. Genet. 6 (2004) 579–581.
[23] O. Topaz, R. Bergman, U. Mandel, G. Maor, R. Goldberg, G. Richard, E. Sprecher, Absence of intraepidermal glycosyltransferase ppGalNac-T3 expression in familial tumoral calcinosis, Am. J. Dermatopathol. 3 (2005) 211–215.
[24] Y. Frishberg, O. Topaz, R. Bergman, D. Behar, D. Fisher, D. Gordon, G. Richard, E. Sprecher, Identification of a recurrent mutation in *GALNT3* demonstrates that hyperostosis-hyperphosphatemia syndrome and familial tumoral calcinosis are allelic disorders, J. Mol. Med. 1 (2005) 33–38.
[25] S. Ichikawa, V. Guigonis, E.A. Imel, M. Courouble, S. Heissat, J.D. Henley, A.H. Sorenson, B. Petit, A. Lienhardt, M.J. Econs, Novel *GALNT3* mutations causing hyperostosis-hyperphosphatemia syndrome result in low intact fibroblast growth factor 23 concentrations, J. Clin. Endocrinol. Metab. 5 (2007) 1943–1947.
[26] H. Olauson, T. Krajisnik, C. Larsson, B. Lindberg, T.E. Larsson, A novel missense mutation in *GALNT3* causing hyperostosis-hyperphosphataemia syndrome, Eur. J. Endocrinol. 6 (2008) 929–934.
[27] R.E. Melhem, S.S. Najjar, A.K. Khachadurian, Cortical hyperostosis with hyperphosphatemia: a new syndrome? J. Pediatr. 6 (1970) 986–990.
[28] M.A. Mikati, R.E. Melhem, S.S. Najjar, The syndrome of hyperostosis and hyperphosphatemia, J. Pediatr. 6 (1981) 900–904.
[29] H. Otukesh, R. Hoseini, H. Chalian, M. Chalian, A.E. Safarzadeh, M. Shakiba, A. Poorian, Hyperostosis with hyperphosphatemia and tumoral calcinosis: a case report, Pediatr. Nephrol. 8 (2007) 1235–1237.
[30] Y.A. Talab, A. Mallouh, Hyperostosis with hyperphosphatemia: a case report and review of the literature, J. Pediatr. Orthop. 3 (1988) 338–341.
[31] M.P. Wilson, C.B. Lindsley, B.A. Warady, J.A. Johnson, Hyperphosphatemia associated with cortical hyperostosis and tumoral calcinosis, J. Pediatr. 6 (1989) 1010–1013.
[32] K.G. Ten Hagen, T.A. Fritz, L.A. Tabak, All in the family: the UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases, Glycobiology 1 (2003) 1R–16R.
[33] K.Y. Renkema, R.T. Alexander, R.J. Bindels, J.G. Hoenderop, Calcium and phosphate homeostasis: concerted interplay of new regulators, Ann. Med. 2 (2008) 82–91.
[34] K.E. White, T.E. Larsson, M.J. Econs, The roles of specific genes implicated as circulating factors involved in normal and disordered phosphate homeostasis: frizzled related protein-4, matrix extracellular phosphoglycoprotein, and fibroblast growth factor 23, Endocr. Rev. 3 (2006) 221–241.
[35] S.C. Schiavi, R. Kumar, The phosphatonin pathway: new insights in phosphate homeostasis, Kidney Int. 1 (2004) 1–14.
[36] H. Xu, J.F. Collins, L. Bai, P.R. Kiela, F.K. Ghishan, Regulation of the human sodium-phosphate cotransporter NaP(i)-IIb gene promoter by epidermal growth factor, Am. J. Physiol., Cell Physiol. 3 (2001) C628–636.
[37] O. Hattenhauer, M. Traebert, H. Murer, J. Biber, Regulation of small intestinal Na-P (i) type IIb cotransporter by dietary phosphate intake, Am. J. Physiol. 4 (Pt 1) (1999) G756–762.
[38] I.C. Forster, N. Hernando, J. Biber, H. Murer, Proximal tubular handling of phosphate: a molecular perspective, Kidney Int. 9 (2006) 1548–1559.
[39] D. Prie, V. Huart, N. Bakouh, G. Planelles, O. Dellis, B. Gerard, P. Hulin, F. Benque-Blanchet, C. Silve, B. Grandchamp, G. Friedlander, Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter, N. Engl. J. Med. 13 (2002) 983–991.
[40] L.V. Virkki, I.C. Forster, N. Hernando, J. Biber, H. Murer, Functional characterization of two naturally occurring mutations in the human sodium-phosphate cotransporter type IIa, J. Bone Miner. Res. 12 (2003) 2135–2141.
[41] C. Bergwitz, N.M. Roslin, M. Tieder, J.C. Loredo-Osti, M. Bastepe, H. Abu-Zahra, D. Frappier, K. Burkett, T.O. Carpenter, D. Anderson, M. Garabedian, I. Sermet, T.M. Fujiwara, K. Morgan, H.S. Tenenhouse, H. Juppner, SLC34A3 mutations in patients with hereditary hypophosphatemic rickets with hypercalciuria predict a key role for the sodium-phosphate cotransporter NaPi-IIc in maintaining phosphate homeostasis, Am. J. Hum. Genet. 2 (2006) 179–192.
[42] B. Lorenz-Depiereux, A. Benet-Pages, G. Eckstein, Y. Tenenbaum-Rakover, J. Wagenstaller, D. Tiosano, R. Gershoni-Baruch, N. Albers, P. Lichtner, D. Schnabel, Z. Hochberg, T.M. Strom, Hereditary hypophosphatemic rickets with hypercalciuria is caused by mutations in the sodium-phosphate cotransporter gene SLC34A3, Am. J. Hum. Genet. 2 (2006) 193–201.
[43] S. Ichikawa, E.A. Imel, A.H. Sorenson, R. Severe, P. Knudson, G.J. Harris, J.L. Shaker, M.J. Econs. Tumoral calcinosis presenting with eyelid calcifications due to novel missense mutations in the glycosyl transferase domain of the GALNT3 gene. J. Clin. Endocrinol. Metab. 91(11) (Nov) (2006) 4472–4475.
[44] G. Jaureguiberry, T.O. Carpenter, S. Forman, H. Juppner, C. Bergwitz, A novel missense mutation in SLC34A3 that causes hereditary hypophosphatemic rickets with hypercalciuria in humans identifies threonine 137 as an important determinant of sodium-phosphate cotransport in NaPi-IIc, Am. J. Physiol. Renal. Physiol. 2 (2008) F371–379.
[45] L.A. Brame, K.E. White, M.J. Econs, Renal phosphate wasting disorders: clinical features and pathogenesis, Semin. Nephrol. 1 (2004) 39–47.
[46] T.M. Strom, H. Juppner, PHEX, FGF23, DMP1 and beyond, Curr. Opin. Nephrol. Hypertens. 4 (2008) 357–362.
[47] M. Bastepe, H. Juppner, Inherited hypophosphatemic disorders in children and the evolving mechanisms of phosphate regulation, Rev. Endocr. Metab. Disord. 2 (2008) 171–180.
[48] S.M. Jan de Beur, M.A. Levine, Molecular pathogenesis of hypophosphatemic rickets, J. Clin. Endocrinol. Metab. 6 (2002) 2467–2473.
[49] K. Araya, S. Fukumoto, R. Backenroth, Y. Takeuchi, K. Nakayama, N. Ito, N. Yoshii, Y. Yamazaki, T. Yamashita, J. Silver, T. Igarashi, T. Fujita, A novel mutation in fibroblast growth factor 23 gene as a cause of tumoral calcinosis, J. Clin. Endocrinol. Metab. 10 (2005) 5523–5527.
[50] A. Benet-Pages, P. Orlik, T.M. Strom, B. Lorenz-Depiereux, An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia, Hum. Mol. Genet. 3 (2005) 385–390.
[51] T. Larsson, X. Yu, S.I. Davis, M.S. Draman, S.D. Mooney, M.J. Cullen, K.E. White, A novel recessive mutation in fibroblast growth factor-23 causes familial tumoral calcinosis, J. Clin. Endocrinol. Metab. 4 (2005) 2424–2427.
[52] H. Garringer, M. Malekpour, F. Esteghamat, S. Mortazavi, S. Davis, E. Farrow, X. Yu, D.E. Arking, H.C. Dietz, K.E. White. Molecular genetic and biochemical analyses of FGF23 mutations in familial tumoral calcinosis. Am. J. Physiol. Endocrinol. Metab. 295 (4) (Oct) (2008) E929–937.
[53] S. Ichikawa, E.A. Imel, M.L. Kreiter, X. Yu, D.S. Mackenzie, A.H. Sorenson, R. Goetz, M. Mohammadi, K.E. White, M.J. Econs, A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis, J. Clin. Invest. 9 (2007) 2684–2691.
[54] A.L. Negri, The klotho gene: a gene predominantly expressed in the kidney is a fundamental regulator of aging and calcium/phosphorus metabolism, J. Nephrol. 6 (2005) 654–658.
[55] C.A. Brownstein, F. Adler, C. Nelson-Williams, J. Iijima, P. Li, A. Imura, Y. Nabeshima, M. Reyes-Mugica, T.O. Carpenter, R.P. Lifton, A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism, Proc. Natl. Acad. Sci. U. S. A. 9 (2008) 3455–3460.
[56] O. Topaz, M. Indelman, I. Chefetz, D. Geiger, A. Metzker, Y. Altschuler, M. Choder, D. Bercovich, J. Uitto, R. Bergman, G. Richard, E. Sprecher, A deleterious mutation in SAMD9 causes normophosphatemic familial tumoral calcinosis, Am. J. Hum. Genet. 4 (2006) 759–764.
[57] I. Chefetz, D. Ben Amitai, S. Browning, K. Skorecki, N. Adir, M.G. Thomas, L. Kogleck, O. Topaz, M. Indelman, J. Uitto, G. Richard, N. Bradman, E. Sprecher, Normophosphatemic familial tumoral calcinosis is caused by deleterious mutations in SAMD9, encoding a TNF-alpha responsive protein, J. Invest. Dermatol. 6 (2008) 1423–1429.
[58] C.F. Li, J.R. MacDonald, R.Y. Wei, J. Ray, K. Lau, C. Kandel, R. Koffman, S. Bell, S.W. Scherer, B.A. Alman, Human sterile alpha motif domain 9, a novel gene identified as down-regulated in aggressive fibromatosis, is absent in the mouse, BMC Genomics (2007) 92.
[59] S. Wopereis, D.J. Lefeber, E. Morava, R.A. Wevers, Mechanisms in protein O-glycan biosynthesis and clinical and molecular aspects of protein O-glycan biosynthesis defects: a review, Clin. Chem. 4 (2006) 574–600.
[60] M.A. Tarp, H. Clausen, Mucin-type O-glycosylation and its potential use in drug and vaccine development, Biochim. Biophys. Acta 3 (2008) 546–563.
[61] K. Onitsuka, K. Shibao, Y. Nakayama, N. Minagawa, K. Hirata, H. Izumi, K. Matsuo, N. Nagata, K. Kitazato, K. Kohno, H. Itoh, Prognostic significance of UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-3 (GalNAc-T3) expression in patients with gastric carcinoma, Cancer Sci. 1 (2003) 32–36.
[62] T. Schwientek, E.P. Bennett, C. Flores, J. Thacker, M. Hollmann, C.A. Reis, J. Behrens, U. Mandel, B. Keck, M.A. Schafer, K. Haselmann, R. Zubarev, P. Roepstorff, J.M. Burchell, J. Taylor-Papadimitriou, M.A. Hollingsworth, H. Clausen, Functional conservation of subfamilies of putative UDP-N-acetylgalactosamine:polypeptide N-acetylgalactosaminyltransferases in drosophila, *Caenorhabditis elegans*, and mammals. One subfamily composed of l(2)35Aa is essential in drosophila, J. Biol. Chem. 25 (2002) 22623–22638.
[63] H.H. Freeze, Genetic defects in the human glycome, Nat. Rev., Genet. 7 (2006) 537–551.
[64] K. Kato, C. Jeanneau, M.A. Tarp, A. Benet-Pages, B. Lorenz-Depiereux, E.P. Bennett, U. Mandel, T.M. Strom, H. Clausen, Polypeptide GalNAc-transferase T3 and familial tumoral calcinosis. Secretion of fibroblast growth factor 23 requires O-glycosylation, J. Biol. Chem. 27 (2006) 18370–18377.
[65] Y. Frishberg, N. Ito, C. Rinat, Y. Yamazaki, S. Feinstein, I. Urakawa, P. Navon-Elkan, R. Becker-Cohen, T. Yamashita, K. Araya, T. Igarashi, T. Fujita, S. Fukumoto, Hyperostosis-hyperphosphatemia syndrome: a congenital disorder of O-glycosylation associated with augmented processing of fibroblast growth factor 23, J. Bone Miner. Res. 2 (2007) 235–242.
[66] H.J. Garringer, C. Fisher, T.E. Larsson, S.I. Davis, D.L. Koller, M.J. Cullen, M.S. Draman, N. Conlon, A. Jain, N.S. Fedarko, B. Dasgupta, K.E. White, The role of mutant UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3 in regulating serum intact fibroblast growth factor 23 and matrix extracellular phosphoglycoprotein in heritable tumoral calcinosis, J. Clin. Endocrinol. Metab. 10 (2006) 4037–4042.
[67] M.S. Razzaque, Klotho and Na+, K+-APPase activity: solving the calcium metabolism dilemma? Nephrol. Dial. Transplant. 23 (2) (2008) 459–461.
[68] I. Urakawa, Y. Yamazaki, T. Shimada, K. Iijima, H. Hasegawa, K. Okawa, T. Fujita, S. Fukumoto, T. Yamashita, Klotho converts canonical FGF receptor into a specific receptor for FGF23, Nature 7120 (2006) 770–774.

[69] O.M. Gutierrez, M. Mannstadt, T. Isakova, J.A. Rauh-Hain, H. Tamez, A. Shah, K. Smith, H. Lee, R. Thadhani, H. Juppner, M. Wolf, Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis, N. Engl. J. Med. 6 (2008) 584–592.
[70] D. Rendina, G. Mossetti, G. De Filippo, M. Cioffi, P. Strazzullo, Fibroblast growth factor 23 is increased in calcium nephrolithiasis with hypophosphatemia and renal phosphate leak, J. Clin. Endocrinol. Metab. 3 (2006) 959–963.
[71] Y. Yoshiko, H. Wang, T. Minamizaki, C. Ijuin, R. Yamamoto, S. Suemune, K. Kozai, K. Tanne, J.E. Aubin, N. Maeda, Mineralized tissue cells are a principal source of FGF23, Bone 6 (2007) 1565–1573.
[72] O.I. Kolek, E.R. Hines, M.D. Jones, L.K. LeSueur, M.A. Lipko, P.R. Kiela, J.F. Collins, M.R. Haussler, F.K. Ghishan, 1alpha,25-Dihydroxyvitamin D3 upregulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport, Am. J. Physiol.: Gasterointest. Liver Physiol. 6 (2005) G1036–1042.
[73] F. Perwad, N. Azam, M.Y. Zhang, T. Yamashita, H.S. Tenenhouse, A.A. Portale, Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice, Endocrinology 12 (2005) 5358–5364.
[74] T. Nakagawa, T. Takaiwa, Calcinosis cutis in juvenile dermatomyositis responsive to aluminum hydroxide treatment, J. Dermatol. 9 (1993) 558–560.
[75] D. Fuchs, L. Fruchter, B. Fishel, M. Holtzman, M. Yaron, Colchicine suppression of local inflammation due to calcinosis in dermatomyositis and progressive systemic sclerosis, Clin. Rheumatol. 4 (1986) 527–530.
[76] Y. Ichiki, T. Akiyama, N. Shimozawa, Y. Suzuki, N. Kondo, Y. Kitajima, An extremely severe case of cutaneous calcinosis with juvenile dermatomyositis, and successful treatment with diltiazem, Br. J. Dermatol. 4 (2001) 894–897.
[77] A.J. Chamberlain, N.P. Walker, Successful palliation and significant remission of cutaneous calcinosis in CREST syndrome with carbon dioxide laser, Dermatol. Surg. 9 (2003) 968–970.
[78] X. Qin, M.A. Corriere, L.M. Matrisian, R.J. Guzman, Matrix metalloproteinase inhibition attenuates aortic calcification, Arterioscler. Thromb. Vasc. Biol. 7 (2006) 1510–1516.
[79] H. Cohen, V. Solomon, I.S. Alferiev, E. Breuer, A. Ornoy, N. Patlas, N. Eidelman, G. Hagele, G. Golomb, Bisphosphonates and tetracycline: experimental models for their evaluation in calcium-related disorders, Pharm. Res. 4 (1998) 606–613.
[80] L.P. Robertson, R.W. Marshall, P. Hickling, Treatment of cutaneous calcinosis in limited systemic sclerosis with minocycline, Ann. Rheum. Dis. 3 (2003) 267–269.
[81] A.W. Chung, H.H. Yang, M.W. Radomski, C. van Breemen, Long-term doxycycline is more effective than atenolot to prevent thoracic aortic aneurysm in marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9, Circ. Res. 102 (8) (2008) e73–75.
[82] S. Ichikawa, K.W. Lyles, M.J. Econs, A novel GALNT3 mutation in a pseudoautosomal dominant form of tumoral calcinosis: evidence that the disorder is autosomal recessive, J. Clin. Endocrinol. Metab. 90 (4) (2005) 2420–2423.
[83] P. Spector, J.G. Cooper, M. Indelman, E. Sprecher, Hyperphosphatemic familial tumoral calcinosis caused by a mutation in GALNT3 in a European kindred, J. Hum. Genet. 51 (5) (2006) 487–490.
[84] H.J. Garringer, S.M. Mortazavi, F. Esteghamat, M. Malekpour, H. Boztepe, R. Tanakol, S.I. Davis, K.E. White, Two novel GALNT3 mutations in familial tumoral calcinosis, Am. J. Med. Genet. A. 143 (20) (2007) 2390–2396.
[85] A. Laleye, M.J. Alao, G. Gbessi, M. Adjagba, M. Marche, I. Coupry, I. Redonnet-Vernhet, S. Lepreux, B. Ayivi, R.B. Darboux, D. Lacombe, B. Arveiler, Tumoral calcinosis due to GALNT3 C.516-2A>T mutation in a black African family, Genet. Couns. 19 (2) (2008) 183–192.
[86] A. Corut, A. Senyigit, S.A. Ugur, S. Altin, U. Ozcelik, H. Calisir, Z. Yildirim, A. Gocmen, A. Tolun, Mutations in SLC34A2 cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis, Am. J. Hum. Genet. 79 (4) (2006) 650–656.
[87] M.F. Campagnoli, A. Pucci, E. Garelli, A. Carando, C. Defilippi, R. Lala, G. Ingrosso, I Dianzani, M. Forni, U. Ramenghi. Familial tumoral calcinosis and testicular microlithiasis associated with a new mutation of GALNT3 in a white family. J. Clin. Pathol. 59 (4) (Apr) (2006) 440–442.
[88] A.M. Barbieri, M. Filopanti, G. Bua, P. Beck-Peccoz. Two novel nonsense mutations in GALNT3 gene are responsible for familial tumoral calcinosis. J. Hum Genet. 52 (5) (2007) 464–468.